EFFECTS OF SALT-LOADING ON MELANOTROPES OF THE INTERMEDIATE LOBE OF THE RAT PITUITARY.

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Salt-loading has been shown to have an effect on melanotrope proopiomelanocortin (POMC) mRNA but the mechanism and significance of this effect is not well understood. To further investigate this interaction, Sprague-Dawley rats (n=3) were given 2% saline for drinking water for 2, 5, or 10 days. Control rats received tap water. The levels of POMC mRNA, dopamine D_2 receptor (D_2total) mRNA and D_2 receptor long isoform (D_2long) mRNA were evaluated with image analysis by relative quantification of in situ hybridization experiments using digoxigenin labeled oligonucleotide probes. Preparations showing levels of α-MSH immunoreactivity (IR), intermediate-early gene activity by Fos protein IR, and dopaminergic innervation visualized by tyrosine-hydroxylase IR were likewise quantified by image analysis.

After two days of salt-loading, melanotrope POMC mRNA levels were slightly reduced, by five days significantly reduce, and after 10 days they were 49% of control ($p<0.01$) in consistence with the literature (Pardy et al., Endocrinology 126:2960, 1990). However, inter-animal variabilty was considerable. Contrarily, after 10 days of salt-loading there was a significant increase of α-MSH IR to 125% of control (cf Howe and Thody, J. Endocr. 46:201, 1970). Interestingly, after 10 days of salt-loading, the D_2long mRNA levels were increased to 130% of control. This was a striking result with apparent involvement of all melanotropes. In contrast, in control pituitaries substantial hybridization to the D_2long mRNA was detected in only a subset of melanotropes. After 10 days of salt-loading, the D_2total mRNA was marginally increased in salt-loaded rats as compared to control rats suggesting differential control of D_2 receptor isoforms. The observations of heterogeneous expression of D_2long receptor mRNA and differential regulation of this isoform is consistent with the literature (Chronwall et al. Mol. Cell Neurosci. 5:35, 1994). During salt-loading, the number of melanotropes expressing Fos protein above a set threshold level significantly increased to 158% of control by day 5 and to 214% at day 10.

Since melanotropes are innervated by dopamine-containing axons, we extended the study of effects of salt-loading to include axonal structures. After 5 days of treatment, the percent area covered by tyrosine hydroxylase IR of comparable intermediate lobe sections had significantly increased to 265% of control and after 10 days treatment further to 321%.

Taken together, these results indicate that the control of melanotrope response to chronic osmotic stimulus is a complex process. Differential regulation, rather than down regulation, of D_2 receptor isoforms may play an important role and pre-synaptic parameters appear to be involved as well.